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The potency of the novel tachykinin receptor antagonist CGP49823 at rat and gerbil motoneurones in vitro

Mario F. Pozza [a,*], Hans-Rudolf Olpe [a], Silvio Ofner [a], Catherine Schoeffel [a], Richard H. Evans [b]

[a] *Novartis Pharma Inc., NS Research, CH-4002 Basel, Switzerland*
[b] *Department of Physiology, University of Bristol, School of Medical Sciences, University Walk, Bristol BS8 1TD, UK*

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Abstract

The novel tachykinin receptor antagonist CGP49823 ((2*R*,4*S*)-2-benzyl-1-(3,5-dimethylbenzoyl)-4-(quinolin-4-ylmethylamino)piperidine) has been compared with three other selective non-peptide tachykinin NK_1 receptor antagonists. The drugs were tested as antagonists of the depolarization of spinal motoneurones induced by bath application of the selective tachykinin NK_1 receptor agonist septide-(6–11) (300 nM) for 120 s at 15 min intervals. The antagonists were bath applied and the depolarizations were recorded from lumbar ventral roots of 7 to 12 day old rat and gerbil hemisected spinal cords in vitro. The gerbil preparation is considered to model the human species variant of the tachykinin NK_1 receptor. With the exception of SR140333 ((*S*)-1-[2-[3-(3,4-dichlorophenyl)-1-[[3-(1- methylethoxy)phenyl]acetyl]-3-piperidinyl]ethyl]-4-phenyl-1-azoniabicyclo [2.2.2]octanechloride), the antagonists were approximately thirty-fold more potent on gerbil preparations. The respective mean IC_{50} values from gerbil preparations produced by CP96345 ((2*S*-*cis*)-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl) methyl]-1-azabicyclo[2.2.2]octan-3-amine), CGP49823, SR140333 and CP99994 ((2*S*-*cis*)-*N*-[(2-methoxyphenyl)methyl]-2-phenyl-3-piperidinamine) were, in μM ± S.E. (*n*) 0.10 ± 0.02 (6), 0.22 ± 0.03 (6), 0.30 ± 0.10 (5) and 0.38 ± 0.02 (5) and the corresponding values from the rat preparations were 3.7 ± 0.4 (5), 7.8 ± 1.3 (5), 1.06 ± 0.16 (6) and 10.5 ± 2.2 (7). Dominance of tachykinin NK_1 receptor activity in the measured responses was confirmed by low potency of the tachykinin NK_2-selective antagonist SR48968 ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl] benzamide) which yielded an IC_{50} value of 12.0 ± 2.8 (5) on gerbil preparations and produced less than 50% depression of septide-induced depolarization of rat motoneurones at the highest concentration (100 μM) tested.

Keywords: Electrophysiology; CGP49823; Substance P; Spinal cord; System response; (Rat); (Gerbil)

1. Introduction

CGP49823 ((2*R*,4*S*)-2-benzyl-1-(3,5-dimethylbenzoyl)-4-(quinolin-μ-ylmethylamino)piperidine) is a recently developed tachykinin NK_1 receptor antagonist as characterized by radioreceptor binding assays and functional antagonism in cell and isolated organ assays (Hauser et al., 1994). The functional activity of CGP49823 in a central nervous system preparation has not been systematically examined. The depression of flexor reflexes (Parsons et al., 1996) and excitatory responses of spinal neurones (Radhakrishnan and Henry, 1991) by the tachykinin NK_1 receptor antagonist CP96345 ((2*S*-*cis*)-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl) methyl]-1-azabicyclo [2.2.2]octan-3-amine) suggests that tachykinin NK_1 receptors are functionally active in the spinal cord. Thus in vitro spinal cord preparations are convenient for the analysis of actions of drugs at functional central tachykinin receptors.

Substance P and peptide analogues produce depolarizations that can be recorded from ventral roots. Such depolarizations are dominated by the tachykinin NK_1 receptor population of motoneurones (Fisher et al., 1994). The present study on spinal cord preparations was aimed at providing further information about the tachykinin NK_1 receptor selectivity of CGP49823 in comparison to other antagonists. Tachykinin NK_1 receptors show a species variation in pharmacological properties that was first demonstrated with the selective antagonist CP96345. Our previous work has shown that gerbil and rat preparations

* Corresponding author. Tel.: +41-61-6967782; fax: +41-61-6966142; e-mail: mario_f.pozza@pharma.novartis.com

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possess different variants, with the gerbil showing a higher potency of CP96345 (Lepre et al., 1993) similar to that of the cloned human variant (Fong et al., 1992). Both gerbil and rat preparations have been tested in the present study. The substance P analogue septide-(6–11) (pyr–phe–phe–pro–leu–metNH_2) shows higher selectivity than substance P for tachykinin NK_1 receptors and for this reason it was chosen as the test agonist in the present study.

The tachykinin NK_2 selective receptor antagonist SR48968 ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) (Emonds-Alt et al., 1992) has been included in the present study as a control for the selectivity of the assay towards functional tachykinin NK_1 receptor responses.

2. Material and methods

Experiments were carried out using hemisected spinal cords isolated from 7–12 day old rats (Tif: RAIf (SPF) or gerbils (GE01x (SPF)) essentially as described by Evans (1978). Briefly the rats were decapitated under deep urethane anaesthesia. The spinal cord with attached dorsal and ventral roots (L3–L5) was excised, sagittally hemisected and transferred onto a perfusion block. The preparation was superfused (1 ml/min) with gassed (95% O_2, 5% CO_2) ACSF maintained at 28°C, which contained (in mM): NaCl 120; KCl 2.5; KH_2PO_4 1.25; $CaCl_2$ 2.5; $MgSO_4$ 2; $NaHCO_3$ 30, and glucose 10.

A dorsal root (L3, L4 or L5) was stimulated at two times threshold where threshold was the intensity required to evoke a just discernible response using a silver wire electrode. The corresponding ventral root was placed on a cotton wick Ag/AgCl electrode in order to record depolarizing responses evoked by the agonists. Application of drugs was controlled and all traces were digitized, stored and analyzed using a personal computer and LABVIEW™ programs (National Instruments, USA and New Visions Engineering, Winterthur). Depolarizing responses evoked by septide-(6–11) were recorded from the ventral roots of the lumbal segment. Agonists were applied through the superfusion system for periods of 120 s every 15 min. The actions of test antagonists on responses induced by septide-(6–11) were determined by comparing the amplitudes of the septide-induced depolarizations before and at cumulative 30 min intervals after addition of the antagonist. Quantitative data are given as mean ± standard error of the mean (S.E.M.). Significance of differences between means for the IC_{50} values was estimated using Kruskal–Wallis nonparametric Anova test.

3. Results

Concentration effect plots were produced with septide-(6–11) and also with substance P on some preparations so that the action of septide-(6–11) could be compared with data from previous experiments using substance P (Lepre et al., 1993). Typical concentration-dependent depolarizing responses of rat and gerbil preparations to bath applied septide-(6–11) are shown in Fig. 1A and B.

The range of depolarizing response amplitude in rat and gerbil preparations was similar to that observed previously using substance P as agonist (Lepre et al., 1993) although as illustrated in Fig. 1C the concentration effect profile for septide-(6–11) was steeper than that for substance P. The concentration of septide-(6–11) (300 nM) chosen for the comparison of antagonist potencies produced a mean depolarizing response of 1.03 mV ± 0.37 ($n = 20$) in the rat preparation and 0.80 mV ± 0.22 ($n = 20$) in the gerbil preparation.

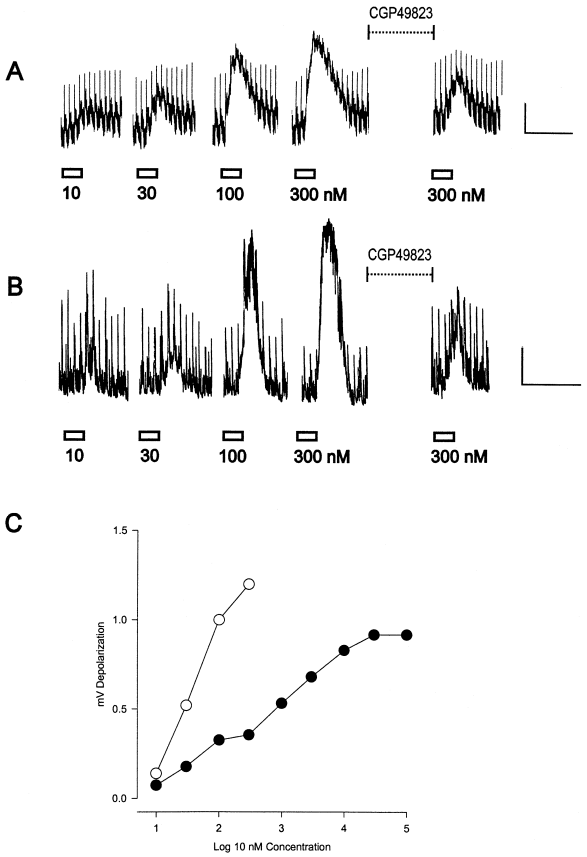

Fig. 1. Action of septide-(6–11) and CGP49823 on electrical activity of spinal cord preparations recorded from ventral roots. Depolarizing responses to 2 min applications of septide-(6–11) are shown in A for a rat preparation and B for a gerbil preparation. The regular vertical deflections are the synaptic responses in the ventral root to electrical stimulation of the dorsal root at 60 s intervals. Septide-(6–11) was applied at the nanomolar concentrations indicated below the traces. The breaks on the right in A and B indicate an interval of 2 h during which CGP49823 was applied. The concentration of CGP49823 for the rat (A) was 10 μM and for the gerbil (B) 300 nM. The subsequent responses to septide-(6–11) were depressed but the synaptic responses to dorsal root stimulation were unaffected. (C) A concentration effect plot of depolarizations induced in the same rat preparation by septide-(6–11) (open circles) and substance P (filled circles). The scale bars on the right of (A) and (B) represent 0.4 mV and 10 min.

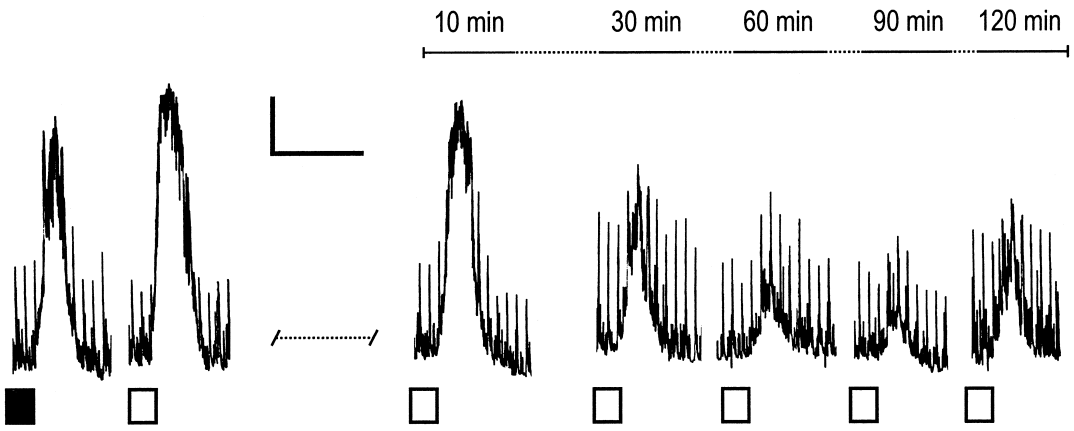

Fig. 2. Time-course of antagonism of septide-(6–11) induced depolarizations in a gerbil preparation. Septide-(6–11) (100 nM (■) and 300 nM (□) was introduced for 2 min as indicated by each square. The vertical deflections are synaptic responses as seen in Fig. 1A and B. In addition spontaneous synaptic activity of approximately 10% of the amplitude of the dorsal root-evoked responses can be seen in the intervals between the evoked synaptic responses. CGP49823 (300 nM) was introduced during the large break in the trace following the second septide-induced response. The following five responses were recorded at intervals of 10, 30, 60, 90 and 120 min after introduction of CGP49823. The depressant action of CGP49823 increased progressively up to 90 min. It can be seen that the dorsal root-evoked and spontaneous synaptic responses were not significantly depressed by the antagonist. The scale bar on the left represents 0.4 mV and 10 min.

A period of application in excess of 60 min was required in order to attain a stable depression of septide-induced responses. This is illustrated for CGP49823 in Fig. 2 where there was a progressive depression of the septide-induced response measured at intervals during application of the antagonist over a period of 120 min. Therefore, for the comparison of antagonist potency a period of two hours application of antagonist was allowed in order to attain steady state depression of depolarizing responses. The antagonism following two hours treatment with 300 nM CGP49823 is illustrated in Fig. 1A and B. The depressant effect of the antagonist can be seen to be greater on the gerbil preparation (Fig. 1B).

In all preparations tested measurement of the peak amplitude of the population EPSP, as reflected by the regular vertical deflections in the traces of Fig. 1A and B and Fig. 2, showed that CGP49823, at concentrations that produced clear depression of septide-induced responses, had little or no effect on dorsal root-evoked synaptic activity. Visual inspection of recordings like those in Figs. 1 and 2 also revealed no obvious depressant action on spontaneous synaptic activity. This lack of effect on synaptic activity is illustrated in the right hand traces in Fig. 1A and B and in Fig. 2.

In order to measure IC_{50} values for antagonism, depolarizing response amplitude, as a percentage of control response amplitude obtained before application of antagonist, was plotted against the antagonist concentration. The concentration of antagonist was increased cumulatively with 90 min application of each concentration. The plots shown in Fig. 3 show that the relative antagonist potencies of CP96345, CP99994 ((2*S*-*cis*)-*N*-[(2-

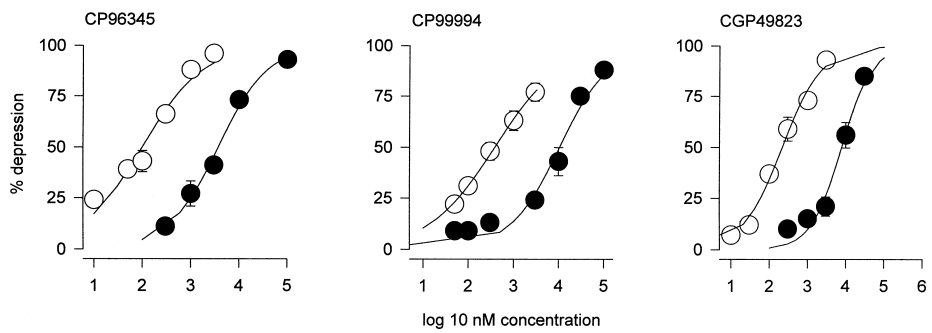

Fig. 3. Concentration effect plots showing antagonism of depolarizations induced by 100 nM septide-(6–11) on gerbil (○) and rat (●) preparations. Respective antagonists are indicated at the top left of each panel. A least squares regression line has been plotted through each set of points using the expression $Y = 100 \times XS/(IC_{50} + XS)$ where S = slope factor. The Y axes show percentage depression of control responses and log 10 nM concentration of antagonist is shown on the X axes. The bars indicate S.E. of mean responses from at least three preparations. Some of the bars are concealed by the symbols.

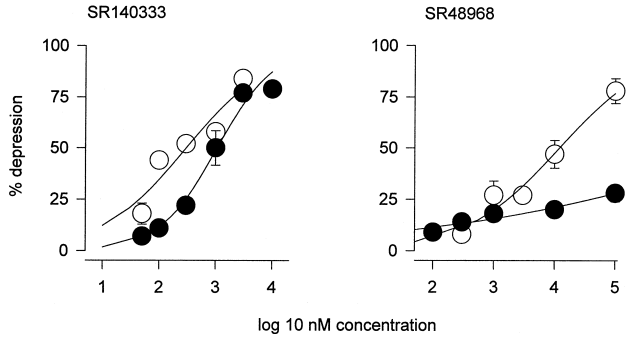

Fig. 4. Concentration effect plots showing antagonism of depolarizations induced by 100 nM septide-(6–11) on gerbil (○) and rat (●) preparations. All other details as for Fig. 2.

methoxyphenyl)methyl]-2-phenyl-3-piperidinamine) and CGP49823 on rat and gerbil preparations was remarkably similar. In each case the plot for the rat preparation is displaced to the right of the plot for the gerbil preparation. The septide-induced depolarizations of gerbil motoneurones (open circles) were about thirty-fold more sensitive to CP96345, CP99994 and CGP49823 than were those of the rat (filled circles).

In contrast, as can be seen from the left hand plot in Fig. 4, there was a less than four-fold difference between rat and gerbil preparations in sensitivity to SR140333 ((*S*)-1-[2-[3-(3,4-dichlorophenyl)-1-[[3-(1- methyl-ethoxy)phenyl]acetyl]-3-piperidinyl] ethyl]-4-phenyl-1-azoniabicyclo[2.2.2]octanechloride).

As an index of tachykinin NK_1 receptor selectivity the tachykinin NK_2 receptor selective antagonist SR48968 was included in the present experiments. It can be seen from the right hand plot in Fig. 3 and the values in Table 1 that this drug had a potency at least thirty-fold lower than the other drugs. The rat preparations showed a very low sensitivity to SR48968, failing to yield an IC_{50} at the highest concentration (100 μM) applied.

Mean IC_{50} values and slope factors from the plots of Figs. 2 and 3 are given in Table 1. It can be seen from Table 1 that all the antagonists except SR140333 showed the same rank order (CP96345 > CGP49823 > CP99994 > SR48968) of potency in the two species. However, the IC_{50} values for CP96345, CP99994 and CGP49823 obtained from gerbil preparations were not significantly ($P > 0.05$) different. Similarly, the mean IC_{50} values for these three antagonists obtained from rat preparations were not significantly different.

Except for CP96345 in the gerbil preparation (upper 95% confidence limit = 0.95) and SR48968 (upper 95% confidence limit = 0.79 gerbil and 0.24 rat) the mean slopes of the concentration effect plots in Figs. 2 and 3 presented in Table 1 are not significantly different from unity.

4. Discussion

The present investigation consists of measurements of the depression of depolarizing responses of motoneurones as recorded from spinal ventral roots. Such depolarizing responses will include, in addition to the component from direct activation of tachykinin receptors on motoneurones, an indirect component synaptically relayed to motoneurones from both excitatory and inhibitory interneurones excited by the bath applied agonist. The latter component may be avoided by including tetrodotoxin in the bathing medium in order to block conduction. We did not employ tetrodotoxin for this purpose in the present investigation. However, a previous study in our laboratory showed that the indirect component, as estimated from sensitivity to tetrodotoxin, amounted from 30% to 40% at low concentrations of agonist to an undiscernible amount near the top of the concentration–effect plot (Lepre et al., 1994). Since the concentration of septide used in the present experiments was near the top of the concentration–effect plot (Fig. 1) we believe that the measured responses were dominated by tachykinin receptors of motoneurones. Fisher et al. (1994) also have found with a similar preparation that the responses to bath applied agonist were dominated by tachykinin NK_1 receptors of motoneurones. However, our experiments do not exclude the possible participation of a small component due to tachykinin NK_1 receptors present on interneurones. This possibility is unlikely to be important in terms of our characterization of the pharmacological selectivity of the present antagonists since our use of the selective agonist septide and our results with the tachykinin NK_2 antagonist SR48968 show that we are dealing with a homogeneous population of tachykinin NK_1 receptors. Certainly the slopes of the concentration–effect plots are consistent with this conclusion.

Table 1
Mean ± S.E. IC_{50} values (slope factor) (*n*) for antagonism of septide-induced depolarizations of motoneurones

Antagonist	Gerbil			Rat		
	mean ± S.E. (μM)	slope factor	*n*	mean ± S.E. (μM)	slope factor	*n*
CP96345	0.10 ± 0.02	0.69 ± 0.10	6	3.70 ± 0.4	0.85 ± 0.08	5
CP99994	0.38 ± 0.02	0.59 ± 0.19	5	10.50 ± 2.2	0.79 ± 0.14	7
CGP49823	0.22 ± 0.03	0.84 ± 0.08	6	7.80 ± 1.3	1.10 ± 0.18	5
SR140333	0.30 ± 0.10	0.58 ± 0.15	5	1.06 ± 0.16	0.86 ± 0.10	6
SP48968	12.0 ± 2.8	0.57 ± 0.08	5	> 100	0.16 ± 0.03	5

The values are calculated from the data used to plot Fig. 2Fig. 3 using the expression $y = 100 * x^2/IC_{50} + x^s$) where s = slope factor.

The present study confirms that tachykinin NK_1 receptors of gerbil motoneurones are more sensitive to CP96345 than those of the rat. Lepre et al. (1993) found a hundred-fold difference in antagonist potency of CP96345 when substance P was used as the agonist. In the present study with septide-(6–11) as the agonist, the difference was thirty-seven-fold. The difference between the two studies is unlikely to be significant since pA_2 was used in the former and IC_{50} in the present study as the measure of antagonism. The present data with CP96345 (mean IC_{50} 0.1 μM) at gerbil motoneurones corresponds with values obtained previously in ours and other laboratories and even for other regions of the CNS. E.g. Lepre et al. (1993) obtained an apparent K_D of 0.16 μM (antilog of pA_2) for antagonism at tachykinin NK_1 receptors whereas Beresford et al. (1994) in experiments at rat spinal motoneurones obtained a value that equates to 74 nM. McLean et al. (1991) obtained an IC_{50} value of 90 nM for antagonism at locus coerulus neurons of guinea pig brain. In the study by Beresford et al. (1994), CP96345 was fifty-five times more potent at displacing labeled substance P from binding sites when membranes were prepared from gerbil rather than from rat brain.

The new antagonist CGP49823 showed an antagonist profile similar to that of CP99994 and CP96345 (Fig. 2). Thus all three antagonists show the same pattern of discrimination between the tachykinin NK_1 receptor species variants in these two species.

It may be significant that these three antagonists, unlike the tachykinin NK_2 antagonist, possess one or more secondary amino groups. CP9994 has the simplest chemical structure amongst the present antagonists (C_{19} compared to at least C_{28}). It is surprising that introduction of an additional bulky quinolyl moiety as in CGP49823 produced no significant change in potency. It seems that the binding site must have a high degree of steric tolerance.

CP96345 has non-selective actions at voltage-sensitive channels (Caesar et al., 1993) as identified by sensitivity to Verapamil. However, the affinity of CP99994 for verapamil binding sites is a hundred-fold lower than that of CP96345 (McLean et al., 1993). In the present study there was only a three-fold difference in antagonist potency between CP96345 and CP99994 (Table 1). Thus non-selective effects on ion channels were unlikely to have been involved in the present measurements.

Non-selective effects of the antagonists would have been revealed through effects on the dorsal root evoked responses which are entirely glutamatergic in the present preparation being abolished by ionotropic glutamate receptor antagonists (Long et al., 1990). Indeed previous experiments in our laboratory have failed to reveal direct depressant actions of tachykinin receptor antagonists on synaptic responses recorded from ventral roots although such synaptic responses can be potentiated by bath applied tachykinin and the tachykinin-induced potentiation can be selectively reversed by antagonists (Lepre et al., 1993).

Septide-(6–11) was chosen as the selective agonist for the present study. This choice could be criticized because septide-(6–11) has been considered to bind to a separate site of the tachykinin NK_1 receptor to substance P (see Glowinski, 1995). The potency of a competitive non-peptide antagonist appears to be independent of which of these agonists is used (Gauchy et al., 1996). This is not surprising when it is considered that the affinity of CP96345 for recombinant tachykinin NK_1 receptor was altered dramatically by remote alterations in receptor structure (Fong et al., 1992) indicating that an apparently competitive antagonist could have a remote effect on agonist binding sites. Comparison of our present data with a previous study (Lepre et al., 1993) shows that a similar pattern of antagonism emerges, as reflected by the potency of CP96345, when substance P was used as the agonist on rat and gerbil preparations. Thus species difference appears to affect the antagonist rather than the agonist potency. The tachykinin NK_1 selective agonist GR73632 (*N*-(5-amino-1-oxopentyl)-L-phenylalanyl-L-phenylalanyl-L-prolyl-*N*-methyl-L-leucyl-L-methioninamide) was found to be thirty-fold less potent as an aversive agent in the gerbil compared to the mouse following intrathecal application (Smith et al., 1994). It would be interesting to see if this agonist also yielded a similar profile with the present tachykinin NK_1 receptor antagonist.

Previous reports from single cell studies, some from our own laboratories, appear to contrast with the present data. For example CP96345 (10 μM) failed to antagonize substance P-induced depolarization of single motoneurones (Lepre et al., 1996). Also SR140333 (1 to 10 μM) did not antagonize substance P- or septide-induced depolarization of rat dorsal vagal neurones (Martini-Luccarini et al., 1996). However, in a different study SR140333 (1 μM) did selectively antagonize tachykinin NK_1 receptor agonist induced depolarization of rat motoneurones (Baranauskas et al., 1995). These apparent anomalies are probably explained by the long time course required for the antagonists to attain equilibrium (Fig. 2). Thus it would be difficult to observe full antagonism because of the problem of maintaining single cell access for at least 60 min.

SR140333 had a profile very different to the other four antagonists in that there was a less than four-fold difference in IC_{50} for antagonism of septide-induced responses between the two species. This corresponds to an approximately two-fold lower K_i value of SR140333 for displacement at human compared to rat tachykinin NK_1 receptors (Regoli et al., 1994). It appears therefore that SR140333 does not discriminate so easily as the other antagonists between the species variants of tachykinin NK_1 receptor. Unlike the other drugs in the present study, SR140333 is a quaternary ammonium compound. This is likely to be highly significant in explaining the different profile of activity of SR140333. It would be interesting to investigate a quaternary version of CP9994.

The low potency and non-competitive slope in Fig. 3

for the tachykinin NK_2 antagonist SR48968, compared to the behavior with the other antagonists, confirms that the depolarizations measured in the present measurements were mediated at tachykinin NK_1 receptors. The failure to obtain an IC_{50} value for SR48968 in rat preparations is consistent with concentration–effect plots for substance P and the tachykinin NK_2 selective agonist [β-ala^8]neurokinin A-(4–10) that showed the equipotent molar potency ratios (substance P/[β-ala^8]neurokinin A-(4–10)) of these two agonists to be ten-fold greater in rat than in gerbil preparations (Lepre et al., 1994).

With the exception of the values for SR48968 the slopes of the concentration effect plots (Table 1) are consistent with binding of the antagonists to a single saturable site.

In conclusion the results show that with septide-(6–11) as agonist the species difference in tachykinin NK_1 receptor antagonist potency at motoneurones was similar to that previously observed with substance P as agonist (Lepre et al., 1993), the tachykinin NK_1 receptor antagonists being much more potent in the gerbil preparation than in the rat. Thus septide-(6–11) appears to activate a population of tachykinin NK_1 receptors on spinal motoneurones indistinguishable to that activated by substance P. The new agent CGP49823 had a similar antagonist profile in the present functional test to CP96345 and CP99994. Thus the present results confirm the tachykinin NK_1 receptor selectivity of CGP49823 (Hauser et al., 1994) in a functional test in the central nervous system.

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